HISTORY OF THE GERM CELLS AND EARLY EMBRYOLOGY OF CERTAIN APHIDS

A DISSERTATION

SUBMITTED TO THE FACULTY OF THE OGDEN GRADUATE SCHOOL OF SCIENCE IN CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

(DEPARTMENT OF ZOÖLOGY)

BY

GEORGE W. TANNREUTHER

CHICAGO
1908

History of the Germ Cells and early Embryology of certain Aphids.

By

Geo. W. Tannreuther.

With plates 49—53.

Contents.

Introduction.

The investigations in the following paper were undertaken for the purpose of studying the development of certain aphids. The significance attached to the development of aphids from different kinds of ova, — sexual and parthenogenetic, — which have the same origin, appearance and fate, the one requiring fertilization and the other not, can not be overestimated. The behavior of these eggs during maturation and the relation of the sexual to the parthenogenetic individuals, afford invaluable material in attempting the determination of sex.

With the importance of these facts in view, I propose to give the history of the aphids upon which the results of this paper are based, paying special attention to the phenomenon of spermatogenesis, ovogenesis and early development.

The work of most previous investigators concerned mostly the habits and life history of the aphids, only incidentally touching the problems of morphology and cytology of the germ cells.

STSCHELKANOVZEW, J. P., 1904, was among the first of the investigators to take up the cytological problem of the aphids. He studied the maturation of the parthenogenetic eggs in *Aphis rosae* and found fourteen chromosomes of various sizes in the prophase of the maturation spindle. After the formation of the polar body, the nucleus contained eight single and three double chromosomes. STEVENS, N. M., 1905, studied the germ cells of *Aphis rosae* and *Aphis oenotherae*. In the maturation of the parthenogenetic eggs of *Aphis rosae*, ten chromosomes of five different sizes were found. This being the somatic number no reduction took place in the formation of the single polar body. In the maturation of the sexual egg, five chromosomes, the reduced number, occurred and both maturation divisions were longitudinal.

Natural history.

The life history of the aphids was studied not only for the purpose of getting a clearer idea of the relation of the parthenogenetic and sexual generations as they occur in nature, but from an experimental standpoint. Individuals from each succeeding parthenogenetic generation were sectioned and studied in order to determine if any morphological or cytological differences occured,

especially in the structure and organization of the germ cells. A careful study proves as far as we are able to determine microscopically that the sex cells or ova of each succeeding parthenogenetic generation are uniform and that no structural differences occur. The somatic number of chromosomes, four large and two small in *Melanoxanthus salicis* and *M. salicicola*, is constant in the ova throughout the entire parthenogenetic generations and in the formation of the single polar body the six chromosomes divide equally.

In order to verifiy these results a number of different species were studied in the sub-families Aphidina and Pemphigina for the purpose of comparison.

The complete life history of only two species *Melanoxanthus salicis* and *M. salicicola* will be given in detail. The habits of both species throughout their entire cycle are very similar, both being found simultaneously on the same host. *M. salicis* is often found on the cottonwood and willow at the same season of the year, but *M. salicicola* is found only on the willow throughout its entire period of existence.

In the spring the stem mother or the first parthenogenetic generation hatches from the winter eggs, which are deposited in the fall. The time for the appearance of the stem mother varies greatly with the condition of the spring season, and the stage of development the embryo reaches in the fall after the eggs are deposited and before cold weather begins. Immediately after hatching the young aphids begin to feed by thrusting their beaks through the bark of the willow twigs and sucking out the sap. They grow very rapidly and moult twice during the first week of their existence. A third moult occurs about the eleventh or twelfth day. At the end of the second week the stem mother begins to reproduce, parthenogenetically. Some individuals observed did not begin reproduction until the twentieth or twenty-fifth day. It requires from five to eight days for the deposition of the forty to seventy-five young aphids by the stem mother. In some instances as in *Pemphigus populi-transversus* a single stem mother may give birth to two-hundred young aphids. The young aphids of the second parthenogenetic generation at the time of birth are completely formed and do not differ in structure and size from the first generation at the time of hatching from the winter eggs. No winged forms are produced in the first generation, but in the second and succeeding

generations a few winged forms appear. There is, however, considerable variation in the number of winged forms in the second generation of *M. salicicola* from a given stem mother; as observed in many instances, ninety-five per cent. of the offspring may become winged. These winged individuals go from the original host to another of the same species and start a new colony. In this way the aphids become more or less evenly distributed in a particular locality. The succeeding parthenogenetic generations agree with the first and second in their habits and structure.

The conditions of food and temperature are a very important feature in aphid development, though they influence only in an indirect way the appearance of the sexual forms. Starting with a given stem mother, favorable conditions promote rapid growth and hasten the reproduction of the succeeding parthenogenetic generations, while unfavorable conditions retard growth and lengthen the time for any given generation.

From a series of observations made in the field and experiments in the green-house, in order to determine the time period for each succeeding generation and the number of parthenogenetic generations that intervened before the appearance of the sexual forms, it was found that external conditions, whether severe or normal, would not bring about the production of the sexual generation before a definite number of parthenogenetic generations intervened. Abundance or scarcity of food is not a factor in determining the sex in case of the aphids. This is shown beyond doubt in the presexual or last parthenogenetic generation where different individuals produce either all males or sexual females irrespective of external conditions.

The minimum period of existence for any given generation in favorable conditions is fifteen days. In unfavorable conditions the maximum period is thirty-five days. If conditions are normal throughout the summer season the average time required for the completion of each parthenogenetic generation is about twenty to twenty-five days. This irregularity for the appearance of succeeding generations is due to food and temperature.

In a number of instances where the host was in an abnormal condition, the required number of parthenogenetic generations (necessary before the appearance of the sexual female) was not produced until the middle of November and very few winter eggs were deposited. In more severe conditions no sexual females were produced at all. Normally, the sexual females appear about the middle of

September after the production of six parthenogenetic generations. These six generations include the stem mother and the presexual generation.

The question naturally arose, — could not the production of the sexual females be brought about earlier in the season with the intervention of fewer parthenogenetic generations? It was found by experimentation that if a stem mother and offspring were kept in favorable conditions in the green-house on the same species of host as out of doors, the time and length of period for each succeeding generation was approximately the same as out of doors and that in both instances the sexual females and males appeared after six parthenogenetic generations intervened. On the other hand, if kept in unfavorable conditions the normal number of parthenogenetic generations intervened before the appearance of the sexual females and males as above. The only differences resulting from the varying of external conditions, are the great irregularities in the appearance of the successive parthenogenetic generations from different stem mothers.

Whether the intervention of six parthenogenetic generations before the appearance of the sexual forms would remain constant or not through a long series of experiments I am unable to say, as the experiments were carried on during two seasons only.

The parthenogenetic and sexual females and males are found coexisting outside from the middle of September until the last of November. If scarcity of food influences the appearance of sexual forms, we would not expect to find some aphids reproducing parthenogenetically so late in the season when the food supply is quite low.

The number of winter eggs that hatch out in the spring is about two per cent. of those that are deposited in the fall, and not more than twenty-five per cent. of those that hatch out reach the adult stage in development. There are no appreciable differences in the appearance or structure of the six parthenogenetic generations.

The presexual generation produces from fifteen to twenty eggs which are like those of the preceding generations. There are no differences in appearance or structure of the ova that produce the parthenogenetic generations and those of the presexual generation that produce the sexual females and males. The behavior of these eggs during maturation and early development is the same in both instances, one polar body being formed without a reduction division.

A very interesting phenomenon occurs in the fifth partheno-

genetic generation where we have the first positive evidence of the beginning of a distinct male and female line or the separation of sex in the parthenogenetic series.

As stated above, the presexual generation, which is produced by the fifth parthenogenetic generation, gives rise to either all male or all sexual female. In the seven generations that complete the life cycle it is found that sex is distinctly separate in two generations only, the presexual (in the sense that male and female, are not produced by the same individual) and in the male and female, also that the union of sex occurs by fertilization at the beginning of the first parthenogenetic generation. Why some of the ova of the fifth parthenogenetic generation produce presexual embryos that give rise only to males, and other ova produce presexual forms that give rise only to sexual females, is a question yet unanswered.

The early embryonic development of the parthenogenetic and sexual generations is very similar, but there are considerable differences in the development of the reproductive systems. In the developing embryo of the parthenogenetic generation the ovarian glands are unspecialized and are often mistaken for primitive ova, as shown in Pl. 50, Fig. 43, and the eggs of the following unborn generation reach the blastoderm stage before the birth of the embryo. On the other hand, in the sexual embryo produced by the presexual individuals, the ovarian glands are specialized (Fig. 30), and have reached their maximum development, while the ova of the unborn sexual developing embryo have not begun to develop at the time of birth.

The winter eggs within the sexual developing individual develop very slowly. The time required from the period of ovulation until the deposition of the winter eggs is approximately the same as that required for the development of a parthenogenetic embryo. This verifies the statement that parthenogenesis is a shortened process for rapid reproductions and lessens the probable fatality which is often due to insect enemies.

Fertilization of the winter eggs occurs just before deposition, but the union of the male and female pronuclei takes place almost immediately after the eggs are deposited. As the eggs are being deposited they are covered by a thin layer of secretion from the accessory glands. At first the secretion is very viscid and has a light color, but on being exposed to the air it becomes very elastic and forms a thin capsule over the entire egg. The eggs are glued

to the willow twigs by means of this sticky elastic capsule. It is very difficult to remove the eggs from the willow twigs immediately after deposition without rupturing them, but after being exposed for twenty-four hours to the air, or after fixation, the eggs can be removed very readily from the willow twigs.

The winter eggs begin to develop immediately after deposition. The embryo passes through the winter in a half-grown condition and is situated within the center of the egg, being completely surrounded by the yolk. Growth is completed in the following spring. There are, however, exceptions to this rule. Embryos need not reach half their normal size in the fall in order that growth may be continued in the following spring. If the embryo has reached the germ band stage, i. e. when the germ bond is completely separated from the serosa (Pl. 51, Fig. 51) and lies completely within the center of the egg, growth may be completed in the following spring. But, on the other hand, when the blastoderm is completely formed and only partly invaginated, the embryo does not survive the winter. A very small per cent. of the late deposited eggs develop in the following season. Eggs deposited on willow twigs in the green-house begin to develop like those in favorable conditions out of doors, but do not hatch out. Those brought into the green-house immediately after one or several freezings will hatch out. Thus it appears that freezing is necessary for the completion of development.

In studying the life history of the aphids we find that parthenogenesis and the determination of sex are directly related. It is quite evident that fertilization does not play a direct role in the determination of sex as found in some of the Hemipterean insects where the chromosomes vary in size and number in the different germ cells. If it did we would expect to find two distinct parthenogenetic lines beginning at the time of fertilization of the winter eggs. In the one the paternal characters would dominate throughout the parthenogenetic series and give rise to males only in the fall of the year when the sexual generation appears. In the other, the female characters would dominate and give rise to sexual females only. We were unable to find a single instance where a given stem mother gave rise directly to either a male (paternal) or female (maternal) line. The first evidence we do have of sex being separated, i. e., a dominance of either male or female characters, occurs in the fifth parthenogenetic generation where a single parthenogenetic individual produces presexual forms that give rise to either all

males or females, i. e., males and females are not produced by the same presexual parent. We can not consider the individuals of the fifth parthenogenetic generation as sex hybrids with either male or female characters dominant. If either were true, it would be difficult to account for the production of both sexes under the same conditions. Nor can we consider the parthenogenetic forms (CASTLE, 1903) as sex hybrids in which there is a uniform dominance of the female character. Were this the case, it would be equally difficult to account fo the appearance of males, as the parthenogenetic eggs which produce the males have but one polar body formed and the female character which dominates would not be eliminated.

The view is presented by (STEVENS, 1905) that in parthenogenetic eggs which undergo no reduction, dominance of sex character may be reversed by external conditions. This view can not be applied in the case of the aphids I have studied. If it were true we would not have the appearance of both male and female under like conditions at the same season or time of the year, as occurs in the species studied.

There are no differences in the size and structure of the ova which produce males and females. The number and form of chromosomes are alike in both instances. We could hardly conceive that the sex determinant is found in any particular chromosome. If it is, its behavior is the same in the parthenogenetic eggs that produce either male or female. It is possible that the distribution or division of the chromosomes in the formation of the polar body, which may be qualitatively different, plays the role of sex determinant. Just what the determining factor is, as to whether a parthenogenetic egg shall produce a male or female under the same external conditions can only be conjectured, but the weight of evidence seems so indicate that the determination of sex is due to a structural difference in the chromosomes.

As stated above, the chromosomes are constant in size and number, but vary in the number and size of chromomeres for each chromosome. Again it seems possible that the chromosomes may perform a definite and special function in sex production without being in themselves qualitatively or quantitatively different, except in the degree of their special activity. This later view is suggested very forcibly from the fact that the completely formed chromosomes are alike in number and size in the male and female producing ova.

History of the germ cells.

1. Spermatogenesis.

The history and formation of the germ cells of the successive generations is one of the most striking features in the development of the aphids. They are the first cells differentiated and are recognizable when the blastoderm begins to invaginate at the posterior pole of the egg. The early stages in the formation of the reproductive organs in the male and female producing forms are indistinguishable.

The adult testes of *Melanoxanthus salicis* are paired, each part consisting of five more or less radially arranged lobes. The blind end of each lobe is larger than the proximal end, which tapers down and becomes more tubular. The five lobes unite at a common point and form the vas deferens, which meets its fellow on the opposite side. Each lobe is composed of a number of cysts. In Pl. 51, Fig. 45, the longitudinal section of a single lobe shows eight cysts, containing sex cells in different stages of development ranging from the growth period to the nearly completely developed sperm. The cells of any individual cyst vary slightly in their stages of development.

The cells of the last spermatagonial division are composed of a clear homogeneous mass of naked protoplasm. The nucleoplasm is clearer and less granular than the cytoplasm. At the beginning of the last spermatogonial division (Pl. 53, Fig. 62—63), the chromatin collects at definite points on the spireme (Fig. 64), and forms six distinct chromosomes, four large and two small (Fig. 65—67). This difference in size of chromosomes is a constant feature throughout spermatogenesis. The chromosomes arrange themselves in an equatorial plate (Fig. 68). The different phases in the last spermatogonial division are shown in Fig. 69—73. The division of the nucleus and cytoplasm is not always simultaneous. In some instances the daughter nuclei are in late telophase before the cytoplasm begins to divide (Fig. 73). The six chromosomes are distinct at the close of the last spermatogonial division, but immediately at the beginning of the growth period they lose their individuality, assume a granular condition (Fig. 75), and collect in a single homogeneous mass within the center of the nucleus. The chromatin remains in this condition during the growth period (Fig. 75—76). In the early prophase of

the first spermatocyte division the chromatin granules collect at definite points and form a distinct spireme (Fig. 77—78). The spireme shortens (Fig. 78—80) and the chromatin masses at different points on the spireme (Fig. 81—83). This process continues until the six definite chromosomes are formed (Fig. 84—86). There is no nuclear membrane formed but the nucleoplasm is distinctly clearer than the cytoplasm. There is apparently no fusion of chromosomes during the growth period. The somatic number six is found in the prophase of the first spermatocyte division. The chromosomes unite end to end in the prophase of the first spermatocyte division (Fig. 87—88). The four large form two pairs, and the two small chromosomes one pair. These fused pairs of chromosomes have the same characteristic shape as the single chromosomes except that they are larger (Fig. 89). The three bivalent chromosomes are arranged in an equatorial plate and the nuclear and cytoplasmic areas are distinct (Fig. 90). There is no definite achromatic spindle formed, but in the early metaphase (Fig. 91), as the chromosomes divide, they remain connected at a common center or point by thread-like fibers, which have the same affinity for stain as the chromatin (Fig. 92). In passing from the metaphase to the telophase these fibres become more united and form a common interzonal mass of fibres (Fig. 93—96). In the late telophase these fibres loose their immediate connection with the chromosomes (Fig. 95), but persist in the cytoplasmic area after the spermatocytes have completely divided (Fig. 97). There is a short resting stage before the second spermatocyte division (Fig. 98). The first maturation or spermatocyte division separates paired chromosomes. The spermatocytes of the second generation have three chromosomes, two large and one small (Fig. 99). In the second spermatocyte division, the interzonal fibres exist as in the first, but play no part in the formation of the sperm. The early transformation stages of the spermatids before the cytoplasm begins to elongate are shown in Fig. 107—110.

The nucleolus first appears after the last spermatogonial division at the beginning of the growth period. It is found in its earliest stage in the center of the chromatin area (Fig. 76). It has the same affinity for stain as the completely organized chromosomes. Before the chromosomes become distinct the nucleolus passes to the periphery of the nucleus and divides (Fig. 82). Or as in some instances, it divides before passing to the periphery. The nucleolus

appears only in the growth period and early prophase of the first spermatocyte division. In the ova of the same species nucleoli appear in the germinal vesicle during the growth period and disorganize within the nucleoplasm when the polar bodies are formed.

Degenerating cells are found in the cysts of the different lobes of the testes. Seldom more than two cysts in a single lobe are affected. These degenerating cells are found at the close of the last spermatogonial division and in the prophase of the first spermatocyte division. Degeneration in the first instance is shown by a failure of the chromosomes to pass into the granular condition at the beginning of the growth period as occurs in the normal cells. The chromosomes instead fuse in a common mass, which stains very dark. The cytoplasm becomes very clear, more granular and almost entirely disappears, leaving a faint ring of cytoplasm around the dark chromatin mass (Fig. 111—112).

In the second instance the chromatin spireme, instead of forming the distinct chromosomes, becomes a homogeneous mass (Fig. 113). This fused nuclear mass often divides within the cytoplasm and gives the appearance of a multinucleate cell (Fig. 114—116). Wilcox, 1895, in *Cicada tibicen* and *Caloptenus femur-rubrum* found that degeneration and amitosis as well affected only the spermatogonia.

2. Ovogenesis.

a) Parthenogenetic generation.

The reproductive organs consist of ten follicles in two groups. Each follicle is distinctly divided into three parts, end ligament, ovariole or end chamber, and oviduct. The oviducts of both sides lead to a common uterus. The structure and appearance of a single follicle as shown in Pl. 50, Fig. 43, gives the exact relation of the three parts or divisions. The end chamber is almost completely filled with the nutritive or ovarian glands. Two ova are plainly visible at the proximal end of the end chamber. The ova just before entering the oviduct are connected with the inner ends of the ovarian glands by the nutritive string. The polar body is formed immediately after the egg enters the oviduct (Fig. 43). A more advanced stage of an ovum in the early formation of the blastoderm is shown in the oviduct. From five to six embryos in different stages of development may be found in a single oviduct.

The ovogonia at the last ovogonial division are comp(

naked granular mass of protoplasm. The nucleoplasm is clear and finely granular. The chromatin is in a granular condition (Pl. 53, Fig. 117). All of the germ cells that enter the end chamber are potential ova, but all do not develop. This is more particularly true of the ova near the end of any generation. Only one ovum within the end chamber begins development at a time, although two to six ova may be present. Why one ovum within the end chamber at the proximal end begins growth in preference to another is a difficult question to answer. The growth does not begin until the ovum is connected with the nutritive string. This would seem to indicate very strongly that growth is initiated by the nutritive string. The cytoplasmic conditions of the ovarian glands, nutritive string and ova are very similar. LUBBOCK, 1859, maintained that the eggs and nourishing cells were modifications of the epithelium of the end chamber. METSCHNIKOFF, 1866, studied the formation of the end chamber in the viviparous aphids. The end chamber he says arises from a mass of cells in which the more peripheral become the follicular epithelium and the inner cell mass becomes the eggs and nourishing cells. This early interpretation was correct with the exception that the egg cells do not originate from the inner cell mass in common with the nourishing cells or ovarian glands, but originate from the follicular epithelium at the base of the end chamber. BALBIANI, 1870, held that the ovarian glands and ova originate from a special nucleated mass of protoplasm in the center of the end chamber by a process of budding and that the ovarian glands were abortive ova or sister cells of the true ova. Furthermore, he explained the attachment of the eggs to the central cell mass — ovarian glands — of the end chamber by their persistent union with the central cell from which they originated by division. WILL, 1885—1886, considered the ovarian glands of the end chamber as ooblasts, formed by a large nucleus surrounded by a very clear protoplasmic mass and that the nucleus of the ooblast divided into masses of the second order, which became scattered through the protoplasm. Each mass of chromatin is the origin of a vitellogene cell or an epithelial cell and that which remains of the ooblast nucleus becomes the germinal vesicle. These peculiar activities in the ovarian glands, — ooblasts —, as WILL describes are present but their function concerns that of nutrition alone. KORSCHELT, 1886, in his paper on the origin and significance of the different elements, — nutritive, epithelial cells, etc., — says these elements are all

potentially equal in their early history in the formation of the follicles, but become differentiated in their early embryonic development.

The differentiation of a common group of germ cells is well marked in the aphids in the formation of the reproductive organs. The ovarian glands and nutritive string are well developed before the ova enter the end chamber. The condition of the ova at the beginning of the growth period is shown in Fig. 118. The chromatin granules are collected into larger masses. Fig. 119 shows the condition of the germinal vesicle in the center of the egg. The nuclear membrane is not very distinct. As the germinal vesicle moves to the periphery the chromatin collects into six distinct groups (Fig. 120). The nuclear area is very clear, while the cytoplasm is more granular and vacuolated. The wall of the germinal vesicle now breaks down and the nucleoplasm fuses with the cytoplasm. The chromosomes become less granular and stain very dark (Fig. 121). The achromatic activities are not well marked, but the chromosomes become arranged in an equatorial plate (Fig. 122) and divide equally, forming a single polar body (Fig. 123—123). Six chromosomes, the somatic number, are found in the polar body and cleavage nucleus. There is no reduction in the formation of the polar body. The polar body does not pass out of the egg, but remains near the periphery in the cytoplasm. It stains as a dense homogeneous mass and persists after cleavage has begun, but finally disorganizes. The cleavage nucleus passes from the periphery to the center of the egg after maturation, where it enters into a short resting stage.

The maturation of the parthenogenetic eggs is very interesting on the account of its bearing on the theory of fertilization by the second polar body and the individuality of the chromosomes. The six chromosomes, four large and two small, are constant throughout parthenogenetic reproduction and divide equally in the formation of the single polar body. The number of parthenogenetic generations does not affect the usual course in the maturation of the fertilized eggs. The idea that the second polar body determines the male sex has no significance in the parthenogenetic eggs of the aphids. MINOT, 1877, suggested that parthenogenesis may be due to the failure to form polar bodies, i. e., the entire chromatin remained within the egg, — hermaphroditic, — and is capable of further development without the addition of chromatin from the male cell. BLOCHMANN 1888, found in aphids that the eggs which developed

parthenogenetically only one polar body is formed; in those which required fertilization, two polar bodies were formed and that in insects in general the polar bodies are not thrown off, but that the chromatin mass remains in a vesicle in the protoplasm of the egg near the periphery and are often called polar nuclei.

PETRUNKEWITSCH, 1902, in studying *Apis* found that the eggs deposited by the queen in drone cells never showed any signs of being fertilized. Two polar bodies are always formed. The first polar body always divides with a reduction, half being thrown out of the egg, and the half retained united with the second polar body and formed a "Richtungscopulationskern" with the normal number of chromosomes. This nucleus by three consecutive divisions gives rise to eight cells with double nuclei which ultimately become the testis of the adult drone.

Parthenogenetic eggs from eight different species of aphids were studied for the purpose of comparing the formation and behavior of the polar body. In every instance only one polar body was formed and the chromosomes showed no indication of the formation of a second. The polar body disappears by breaking up into fine granules within the cytoplasm near the periphery of the egg. There is no reduction division in the formation of the polar body. The chromatin behaves as in ordinary cell division. An attempt to explain what stimulates the pronucleus to further development would be purely hypothetical.

b) Sexual generation.

The reproductive organs of the sexual aphids are similar to those of the parthenogenetic. Pl. 50, Fig. 29, represents the condition of a single follicle in a half-grown embryo. Each follicle is divided into an end ligament, end chamber or ovariole and oviduct. The end ligament is a continuation of the distal end of the follicle which tapers down to a capillary calibre and is attached to the dorsal wall of the embryo. The end chamber, often called ovariole or in some instances ovary, is the most important or prominent portion in the embryonic development of the reproductive organs. It originates from a cluster of primitive germ cells, which become separated from the completely formed blastoderm where it first begins to invaginate. The more external cells of this group forms an epithelial membrane which becomes the follicular epithelium of the adult follicle. The epithelial cells placed more distally pro-

liferate very rapidly and form the end ligament. Those at the proximal end also proliferate very rapidly and form the oviduct. The proliferation in the formation of the oviduct continues until it meets the invagination of the hypodermis which forms the vagina. The more interior cells of the cluster that do not contribute to the formation of the end ligament and oviduct cease to divide, become glandular and form the ovarian glands of the end chamber (Fig. 29). These glandular cells in their earlier development are often mistaken for the true ova. The end chamber is almost completely filled with these ovarian glands. The peripheral cytoplasm of these glandular cells becomes vacuolated as growth proceeds. In later development the inner ends of these ovarian glands become connected with the nutritive string (Fig. 30). The condition of the follicle at birth in Fig. 30 represents the ovarian glands as almost completely developed. The follicular walls of the end chamber now become lenticular, except at the extreme proximal end where some of the follicular cells continue to divide and give rise to the ova. Three ova are plainly visible in the lower end of the end chamber. The cytoplasm of the nutritive string has begun to surround the ovum in the center of the other two. The ovum becomes united with the cytoplasm of the nutritive string immediately after growth begins. Very rarely does more than one ovum become connected with the nutritive string at a time. Ova that fail to unite with the nutritive string to not develop, but gradually become disorganized within the cytoplasmic contents of the end chamber. Fig. 31 shows a cross section of an ovum at the time of entering the oviduct at the extreme base of the end chamber. The condition of the follicular wall is plainly represented. Some of the follicular nuclei are in mitoses which give rise to the ova. These potential germ cells continue to pass from the follicular wall into the basal cavity of the end chamber, until the aphid is almost completely grown, or as found in some instances until the beginning of the deposition of the winter eggs. Less than half of the ova that enter the end chamber develop.

The ovarian glands reach their adult condition at the beginning of the period of deposition as shown in Fig. 32. The follicular nuclei at the base of the end chamber no longer divide, and the ova cease to be formed. Fig. 32 shows the exact relation of the ovarian glands, nutritive string and ovum. The true condition of the follicle at the end of deposition of the winter eggs is represented

in Fig. 33. The ovarian glands have begun to disorganize. A completely formed ovum is attached to the nutritive string. Some of the ova that have reached the end of the growth period do not undergo farther development. This condition is found only near the end of deposition when the aphid cease feeding.

The condition of the ovogonia before the last ovogonial division is shown in Pl. 49, Fig. 1—4. The cytoplasm is homogeneous. There is no definite cell wall. The chromatin granules collect and form a spireme (Fig. 2). The spireme divides transversely and forms six chromatin rods, four large and two small (Fig. 3—4). In the late prophase the chromosomes lose their identity and collect in a more common mass in the equatorial plate (Fig. 5), but in the late anaphase the chromosomes are more distinct (Fig. 8). The polar view of the telophase (Fig. 9) shows the reappearance of the two small and four large chromosomes.

Immediately after the last ovogonial division, the ova enter the distal end of the end chamber and begin their period of growth as shown in Fig. 11. Before the ovum become connected with the nutritive string the nuclear area increases more rapidly than the cytoplasmic area. The ova at this stage of development are composed of a naked mass of cytoplasm, slightly granular, and a clearer nuclear area containing the six chromosomes. The chromosomes now break up into chromomeres. The chromomeres of the large and small chromosomes vary in size. Some of the chromosomes show four distinct chromomeres but this number is not always constant.

The chromosomes (Fig. 12) become granular and lose their affinity for stain. When the ovum becomes connected with the nutritive string the cytoplasmic area increases more rapidly than the nuclear area or germinal vesicle. In Fig. 12 the condition of the ovum is represented as it enters the oviduct. The cytoplasmatic granules increases in size and become more variable. The cytoplasm next to the clear nuclear area becomes more dense and has the appearance of a nuclear wall or membrane. As growth continues (Fig. 13) the cytoplasmic area becomes vacuolated and has a slightly granular appearance. The chromosomes are collected into a single homogeneous mass. In the following stage of development (Fig. 14) the ovum becomes more elliptical. The vacuoles of the cytoplasm increase in size and show a more definite reticular condition. The nucleus is in the synapsis stage.

The ovum at the end of the growth period (Fig. 15) shows a

complete reticular condition of the cytoplasm. The yolk granules that are deposited in the reticular spaces grow very rapidly. The peripheral layer of cytoplasm or periplasm is less reticular and does not contain any yolk granules. The nucleus or germinal vesicle is found in the center of the egg with a well organized nuclear wall.

The reduction of chromosomes occurs during the growth period of the ovum. The behavior of the germinal vesicle is very interesting. Starting with the germinal vesicle in the center of the ovum (Fig. 15), the nucleoplasm becomes differentiated into two distinct areas. A very dense granular outer part and a clearer inner part free from granules which contain the chromatin. The chromatin granules become more distinct and form a definite spireme (Fig. 16—17). The germinal vesicle during the process of maturation moves from the center of the egg to the periphery. The spireme thread divides transversely and forms three distinct chromatin rods (Fig. 18—19). The germinal vesicle increases in volume during maturation as it passes to the periphery. The wall of the germinal vesicle does not break down until after it reaches the extreme periphery and the polar bodies are formed.

The achromatic activities of mitosis are absent and the chromosomes act more or less independently although they pass through the same conditions in division as if the true spindle was formed. The chromosomes divide longitudinally in the early prophase of the first maturation division (Fig. 20) and the first polar body is formed (Fig. 21). The second division probably separates univalent chromosomes. The second polar body is represented in Fig. 22—23.

After the formation of the polar bodies the chromatin of the pronucleus becomes granular (Fig. 24) and the wall of the germinal vesicle breaks down. The nucleoplasm fuses with the periplasm around the entire periphery of the egg, and forms the band of cytoplasm which becomes evenly distributed over the entire surface of the egg and later becomes the cytoplasm of the blastoderm cells. The fusing of the nucleoplasm and periplasm is represented in Fig. 25. The polar bodies now appear as two compact masses of chromatin. The pronucleus at this stage has a granular appearance and is scarcely distinguishable. In a tangential section of a little later stage shown in Fig. 26 the polar bodies are still plainly visible and the pronucleus has slightly increased in size and the three chromosomes are in the vesicular condition. This section gives a

better idea as to how the nucleoplasm spreads out over the surface of the egg. In a little later stage than the preceding (Fig. 27), the polar bodies have almost disappeared within the cytoplasm near the periphery of the egg. The pronucleus is somewhat larger but the chromosomes have no longer their vesicular appearance and appear more as a regular granular mass. The pronucleus now moves from the periphery to the center of the egg (Fig. 28). During this passage to the center the pronucleus is surrounded by a scanty mass of cytoplasm. The chromatin remains in the resting condition and it is impossible to detect any chromatic changes.

The male and female pronuclei are represented in Pl. 52, Fig. 53. A little later stage just before fusion is shown in Fig. 53a. The three chromosomes, two large and one small, are shown in each pronucleus. The fusion of the pronuclei occurs immediately after deposition.

The irregularity in the periplasm as seen in Fig. 53 is caused by the nucleoplasm from the germinal vesicle, which has not yet become evenly distributed around the periphery. The interior of the egg is filled with a compact mass of yolk granules and contains but little cytoplasm.

3. General conclusions.

Judging from the early differentiation of the cytoplasm in the ovum during the growth period we can safely conclude that the cytoplasm functionally becomes divided into two separate regions, destined to play a different role in later embryonic development. The outer or peripheral cytoplasm which is slightly reticular and non granular becomes the cytoplasm of the blastoderm cells, while the inner highly granular and reticular cytoplasm with its contained yolk is wholly nutritive.

The prevalent idea that the development of aphids is unstable and controlled directly by external conditions is certainly very misleading, especially the idea that unfavorable conditions or lack of food is a direct cause for the appearance of the winged and sexual forms. We find in the species studied that just the reverse is true and that the greatest number of winged forms are found in the second parthenogenetic generation where in some instances ninety-five per cent. may become winged, especially those found on the host in good conditions which furnished an abundance of food. Why some of the hypodermal cells of the thorax begin to divide, evaginate

and form the adult wings in a few days is not known, but whatever the cause may be, the abundance of food favors their rapid development. In case of the sexual forms scarcity of food retards growth and lengthens the time for each succeeding generation and prevents the appearance of the sexual forms at the usual time, as a definite number of parthenogenetic generations intervenes before the appearance of the sexual forms.

The continuity of the chromosomes, four large and two small, throughout the entire parthenogenetic generations as well as in the sexual generations supports the hypothesis of the individuality of the chromosomes.

The theory that in the parthenogenetic eggs the female character is dominant and eliminated by the second polar body cannot be applied, as but one polar body is formed. The dominance of either male or female character if removed at all must occur in the formation of the single polar body.

One parthenogenetic generation, — presexual, — intervenes after the beginning of the male and female line, before the sexual female and male aphids are produced. Why two parthenogenetic generations are so intimately related in the production of the male and female can not be explained as we were unable to detect any difference in the structure and behavior of the ova. The only differences observable in the generations that are so closely related in the production of the sexual forms is that but few embryos are produced in comparison to the preceding parthenogenetic generations.

Early embryological development.

1. General statement.

The early cleavage and formation of the blastoderm in the parthenogenetic aphids afford nothing of special interest and will be given very briefly, chiefly for the purpose of comparison with the sexual developing embryo. While on the other hand, the early cleavage and formation of the blastoderm in the sexual developing embryo has not been worked out in detail by any previous author to my knowledge, and will be given more in detail.

The mode of development in the sexual embryo differs greatly from that of the parthenogenetic embryo in the same species, but when development is complete in both instances it is impossible to distinguish between the two embryos from external appearances.

2. Development of the ova of the parthenogenetic and presexual generations.

After the formation of the polar body the nucleus passes to the center of the egg. The prophase of the first cleavage nucleus is shown in Pl. 53, Fig. 127—128. The polar body is visible near the periphery as a dark chromatin mass. The spindle of the first cleavage is well organized (Fig. 129). The cytoplasm is vacuolated. After the first cleavage, the daughter nuclei pass nearer the periphery. All the subsequent divisions of the cleavage nuclei occur near the surface of the egg. The position of the cleavage nuclei after the first and second cleavages is shown in Pl. 50, Fig. 34—35. After the third cleavage (Fig. 36—37), the egg becomes more elliptical and the nuclei take a position nearer the surface of the egg (Fig. 38—39). The nuclei divide simultaneously and pass through a short resting stage after each cleavage. The nuclei have a distinct nuclear membrane (Fig. 38). As the egg elongates the cleavage nuclei fuse with the peripheral cytoplasm of the egg. The peripheral cytoplasm separates into parts corresponding to the cleavage nuclei (Fig. 40).

The cleavage nuclei that do not pass to the periphery in the formation of the blastoderm divide once or twice mitotically and prepare the yolk for assimilation. The yolk originates as granules within the cytoplasm. These granules increase in size and form spherical-like masses with yolk nuclei in the center (Fig. 41).

The blastoderm forms a continuous epithelial band around the egg except at the extreme posterior end, where the so-called blastophore is formed. On either side of this opening the blastoderm begins to invaginate by a rapid proliferation of the blastoderm nuclei (Fig. 41—42).

The nuclei of the entire blastoderm may divide once or twice before invagination begins. Thus the blastoderm may become one or several cells thick (Fig. 42).

The blastoderm now has reached its maximum growth and the part which does not invaginate becomes more reticular and forms the serosa.

The development of the ova in the parthenogenetic generations and the presexual or last parthenogenetic is the same. The only difference is that the presexual individuals contain but few ova and

that the ova of the unborn embryo do not leave the ovary until after birth.

Immediately after invagination begins the embryo is provided with a new supply of yolk from the follicular epithelium (Fig. 42). These epithelial cells which produce secondary yolk completely fill the lumen of the oviduct near the posterior end of the developing egg.

It is often very difficult to distinguish between these nutritive cells and the cells of the oviduct wall from which they originate. STEVENS (1905) in *Aphis rosae* describes at the base of the embryo two conspicuous cells which apparently guard a valvular opening in the wall of the oviduct through which the secondary yolk of WILL (1889) passes into the embryo.

This interesting phenomenon of guard cells does not appear in *M. salicis*. The secondary yolk does not pass direct from the walls of the oviduct into the embryo, but pass into the lumen of the oviduct where the nuclei divide and form the yolk. The entrance of the secondary yolk into the embryo is represented in Fig. 42.

3. Development of the ova of the sexual generation.

The first division of the cleavage nucleus occurs in the center of the egg (Pl. 52, Fig. 54). The succeeding cleavages until the sixth occur simultaneously. The descendants from each daughter cell of the first cleavage nucleus contribute to the formation of the blastoderm. According to WEISMANN (1882) in *Rhodites* and *Biorhiza aptera* (*cynipedae*) the cleavage nucleus first divides into two nuclei, one shifting posteriorly and the other anteriorly. The posterior nucleus by division gives rise to nuclei that take part in the formation of the blastoderm. The anterior nucleus, after the completion of the blastoderm, produces by division the nuclei of the so-called inner germ-cells or yolk-cells.

The second and third cleavages are shown in Fig. 55—56. The descendants of each daughter nucleus can be readily traced until after the fifth division. The cleavage nuclei derived from one of the daughter nuclei takes a more posterior position, while those from the other daughter nucleus take a more anterior position in the egg. The cleavage nuclei are surrounded by a small star-shaped mass of cytoplasm. The periphery of the egg shows the narrow band of cytoplasm in which the cleavage nuclei that form the blastoderm finally become imbedded.

The position of all the cleavage nuclei resulting from the four

divisions are represented in Fig. 57. We can readily distinguish the eight nuclei that are situated more posterior from those that are found more anterior. This interesting phenomenon has no special bearing on the later development except that the posterior half of the blastoderm approximately is formed from the offspring of one daughter nucleus, while the anterior half is formed from the offspring of the other daughter nucleus. The final outcome is, since only a very small part of the posterior end of the blastoderm forms the germ band, that the cleavage nuclei resulting from one of the daughter nuclei, that is situated more posteriorly give rise to the entire germ band, part of the serosa and part of the yolk nuclei; while the offspring from the more anterior daughter nucleus gives rise only to a part of the serosa and part of the yolk nuclei.

The formation of the blastoderm begins uniformly over the entire surface of the egg, except at the extreme posterior end which remains almost entirely free from cleavage nuclei while the blastoderm is forming (Fig. 58). The cleavage nuclei pass to the extreme surface of the egg (Fig. 58) and enter the peripheral layer of cytoplasm. The cleavage nuclei that do not pass to the periphery of the egg become active centers for the digestion of the yolk spheres. WHEELER (1889) found in *Blatta* that all the cleavage nuclei passed to the surface of the egg and took part in the formation of the blastoderm, while the center of the egg was devoid of nuclei and that the yolk cells or nuclei appeared later by the wandering of cells from the blastoderm into the interior of the egg.

The point at which cleavage nuclei reach the surface of the egg varies in different groups of insects. In *Muscidae* (GRABER) the formation of the blastoderm begins at the posterior end of the egg. In *Blatta* (WHEELER) the first cells that form the blastoderm appear on the ventral surface, i. e., the future ventral surface of the embryo. In *Pieris* (BOBRETZKY) the blastoderm cells first appear at the anterior end or pole of the egg. In *Hydrophilus* (HEIDER) the blastoderm first forms around the middle of the egg as a transverse girdle somewhat nearer the posterior pole of the egg and develops last at the poles of the egg.

In *M. salicis* the blastoderm begins uniformly over the entire surface of the egg except at the posterior pole. The cleavage nuclei n the formation of the blastoderm divide but once after reaching the surface of the egg (Fig. 59).

The blastoderm before invagination never becomes more than

one cell thick (Fig. 60). When the formation of the blastoderm is complete (Fig. 60), there is a short inactive period and the cytoplasm of the blastoderm now constricts between the blastoderm nuclei and each cell becomes a naked mass of cytoplasm with a nucleus in the center. This constriction of the cytoplasm is only temporary and the blastoderm soon becomes a continuous band of cytoplasm with its nuclei. None of the blastoderm cells except those at the extreme posterior end of the egg take part in the formation of the germ band.

At the close of the short inactive period the cells at the posterir end of the blastoderm begin to divide very rapidly (Fig. 61) and invaginate. The cell walls now become more distinct (Pl. 51, Fig. 49). The condition at the beginning of invagination and the relation of the invaginated part to the secondary yolk is shown in Fig. 50.

The vitelline membrane is not represented in this figure. The yolk digesting nuclei are connected by cytoplasmic cords and thus bring the yolk in direct connection with the developing germ band (Fig. 50). As invagination continues the secondary yolk mass and the cytoplasmic cords connecting the yolk nuclei move toward the anterior pole of the egg (Pl. 52, Fig. 52). This invagination continues until the germ band is entirely within the center of the egg (Pl. 51, Fig. 51). The germ band now becomes separated from the uninvaginated part of the blastoderm, which becomes very lenticular and forms a continuous epithelial layer enclosing the yolk mass with the tubular-like germ band in the center. The germ band now resembles a tube with a uniformly thickened wall. The tubular germ band during invagination is open at either end but closes when invagination is complete and becomes differentiated into two distinct parts or regions. The part which becomes the embryo proper and the part which becomes the amnion (Fig. 51). That part of the blastoderm which invaginates first becomes the posterior end of the embryo, while the part which invaginates last becomes the anterior end.

The secondary yolk which is always found at the posterior end of the egg is very closely related to the invagination of the blastoderm. The blastoderm cells next to the secondary yolk begin to invaginate first. This yolk originates from the follicular nuclei of the oviduct wall which completely fills the lumen of the oviduct next to the posterior end of the egg. Pl. 51, Fig. 46, shows the nuclei passing into the posterior end of the egg. These nuclei after entering the egg divide several times mitotically. The chromatin

breaks up into smaller parts and becomes vesicular. These small vesicles have the appearance of nuclei and may divide. These small vesicles now unite and form common spherical masses (Fig. 47). One or more of these follicular nuclei may contribute to the formation of a single mass. Some of these yolk like spheres do not unite into a common mass, but remain isolated and function as food in the growth period of the embryo. The secondary yolk passes into the interior of the embryo and the part not used up by the embryo as food persists throughout the entire life of the aphid in the body cavity surrounding the reproductive organs. In case of scarcity of food these secondary yolk masses become quite small, and in very severe condition they are almost completely absorbed by the aphid. The primary yolk does not pass direct into the body cavity, but is first changed into a fluid like substance by the yolk nuclei and then taken up by the embryo. The secondary yolk spheres contain chromatin like granules, while the primary yolk spheres are entirely free from chromatin.

4. Comparison and correlation.

The general plan of development in the aphids is similar in the parthenogenetic and sexual generations and differs only in the more minor points.

The origin of the ova from the follicular wall of the oviduct at the base of the end chamber is the same in the parthenogenetic and sexual individuals. The ova of both are composed of a naked mass of cytoplasm with the germinal vesicle in the center. There are six chromosomes, four large and two small. This number is also true of the male cells at the corresponding stage of development. The reduction of the chromosomes in the sexual ova occurs during the growth period. In the male cells reduction occurs in the early prophase of the first spermatocyte division. No reduction occurs in the parthenogenetic ova.

There is a striking difference in the growth period of the ova. In the parthenogenetic forms the growth of the ova continues after the formation of the single polar body and is most rapid during early cleavage. While in the sexual forms the growth of the ova is entirely complete before the formation of the polar bodies. This difference is an adaptation to the conditions external to the ova. In the one instance cleavage occurs while the egg is in the oviduct.

In the other the cleavage does not take place until after the eggs are deposited.

The mode and formation of the nutritive elements are of special interest in the development of the aphid embryo. The most prominent part in the early formation of the reproductive organs is the ovarian glands, which become differentiated before the ova enter the oviduct. Their function is entirely nutritive and become connected with the ova by means of the nutritive string. These nutritive glands are entirely used up and are seldom found in an individual, where all the embryos are completely developed. In the parthenogenetic forms the ovarian glands are poorly developed, while in the sexual they are highly specialized and play an important part in the formation of the primary yolk.

The primary yolk is very scanty in the parthenogenetic ova and when the blastoderm of the developing embryo begins to invaginate the embryo is supplied with the secondary yolk, which enters the embryo at the posterior end of the blastoderm. In the sexual developing eggs the primary yolk is very abundant. The secondary yolk is formed in the posterior end of the sexual egg just before maturation.

The relation of the secondary yolk to the invaginating blastoderm is quite similar in the parthenogenetic and sexual embryos and has the same function in both cases.

The abundance of yolk in the sexual ovum and its scarcity in the parthenogenetic ovum at the beginning of embryonic development does not modify or change the result of the developing embryo. The cleavage and behavior of the cleavage nuclei in both instances are the same.

There is an interesting distinction in the behavior of the invaginating blastoderm. In the sexual developing embryo where we would expect the abundance of yolk to interfere with the invagination, the invagination is more complete, i. e., the germ band before we can detect any differentiation of organs is completely invaginated or immersed within the yolk and separated from the uninvaginated part of the blastoderm which now becomes the serosa. The result is that we have the undifferentiated germ band entirely free within the yolk, which is now completely surrounded by the epithelium or serosa (Pl. 51, Fig. 51). On the other hand, in the parthenogenetic developing embryo with a scarcity of yolk the invagination is less complete, and the uninvaginated part of the blasto-

derm or serosa, amnion and germ band are continuous until after the differentiation of the organs have begun.

The resulting completely developed embryo in both instances is apparently the same, in size, structure and appearance.

The amnion in the parthenogenetic forms can be recognized before the invagination is complete, while in the sexual developing embryo the amnion can not be distinguished until after the germ band is completely separated from the uninvaginated part of the blastoderm and when the differentiation of the germ band begins. The vitelline membrane and chorion are present only in the sexual eggs.

In the adult aphid, where nearly all of the embryos have reached their later stages of development, the reproductive organs undergo a remarkable change. The specialized ovarian glands of the end chamber have entirely disappeared and the follicular wall of the oviduct becomes a thin tough membrane with a few disorganized nuclei.

This change is most marked in the sexual females, where it is very difficult to find the end chamber or ovaries of the reproductive organs after the eggs have reached their growth. The abdomen of the adult female becomes a hypodermal-like sac filled with eggs, being separated only by the thin membranous oviduct wall which can be recognized under high power. This deteriation of the reproductive organs was found in all the species studied, being especially well marked in the sexual generation where the yolk is completely formed before embryonic development begins.

Brief summary of results.

The somatic number of chromosomes, — six —, is a generic characteristic. The chromosomes vary in size, four large and two small. This number and size of chromosomes is constant in both the sexual and parthenogenetic forms.

In the male, the six univalent chromosomes unite end to end in pairs in the early prophase of the first spermatocyte division and form two large and one small bivalent chromosome. There is a short resting period between the first and second spermatocyte division. Each spermatid receives three chromosomes, two large and one small. No accessory chromosome is present. The first division separates bivalent and the second division divides univalent chromosomes.

The six chromosomes at the beginning of the growth period in the sexual ova pass into the resting stage and the reduced number

three, — two large and one small — are found in the prophase of the first maturation division. Both polar bodies are formed before the germinal vesicle breaks down. Fertilization occurs at the time of deposition and the male and female pronuclei unite shortly after the eggs are deposited. Both polar bodies remain within the egg cytoplasm near the periphery and disappear before the beginning of cleavage.

In the ova of the parthenogenetic females the six chromosomes are found in the prophase of the single maturation division. No reduction occurs and the chromosomes divide equally as in the somatic mitoses. The polar body does not disappear immediately as in the sexual ova, but remains within the egg near the periphery as a dark compact mass of chromatin and does not disappear until after the fourth cleavage.

There are no perceptible differences in the sexual and parthenogenetic ova at the beginning of the growth period. They originate from the follicular epithelium at the base of the end chamber.

Cleavage always begins in the center of the egg. The place of division for the subsequent divisions varies. Descendants from both daughter cells of the first cleavage contribute to the formation of the blastoderm. The cleavage nuclei resulting from one daughter nucleus only form the germ band. All of the cleavage nuclei do not pass to the periphery in the formation of the blastoderm. Those that remain within the yolk area aid in the digestion of the yolk and prepare it for assimilation. The blastoderm begins uniformly over the entire surface of the egg. When the blastoderm is completely formed, there is a short inactive period, — sexual embryo.

The uninvaginated blastoderm becomes the serosa. The germ band is completely separated, — sexual, — from the uninvaginated blastoderm. The germ band is of the completely immersed type.

The parthenogenetic embryo is provided with yolk as needed in the process of development.

In the sexual embryo the yolk is completely formed before fertilization.

The sexual males and females develop from parthenogenetically produced ova, while the first parthenogenetic generation develops from sexually produced ova.

The primary yolk originates within the cytoplasm of the egg.

The secondary yolk originates from the follicular nuclei without the egg.

A definite number of parthenogenetic generations are produced before the sexual male and female appear. External conditions do not increase or decrease the number of parthenogenetic generations. The greatest number of winged forms appear in the second generation, especially when the food is abundant. The parthenogenetic developing embryo within the winter or sexual egg passes through the winter in a half-grown condition.

A distinct male and female line begins in the fifth parthenogenetic generation. The individuals of the presexual or last parthenogenetic generation produce either all males or all females. But two generations contribute directly to the formation of the male and female, i. e., the fifth and presexual generations.

Material and methods.

The material upon which this paper is based was collected during the summer and fall months of 1903 and 1904. Different killing reagents were used with varying degrees of success. Corrosive sublimate, acetic acid mixture, gave the best results.

Saturated sol. $HgCl_2$ in 50% alcohol	94 volumes
Glacial acetic acid C. P.	6 „

The killing fluid was heated to 70° C and poured suddenly over the material to be killed. In 5 to 10 minutes the fluid was poured off and replaced by the cool. After killing, the headad part of the thoracic region were removed.

In case of the egg, they were punctured with a fine needle. The material was then dehydrated, cleared in xylol and preserved in paraffine.

The material was sectioned from 6 to 10 μ's thick and stained on the slide. The iron-alum haematoxylin stain gave the best results.

Hull Zoological Laboratory,
The University of Chicago.
July, 1905.

Bibliography.

1. Ashmead, Wm. H., 1890, A generic synopsis of the Aphididae, in: Entomol. Americana, Vol. 5, No. 10/11, p. 183—189.
2. Balbiani, E. G., 1882, Sur la signification des cellules polaires des insectes, in: CR. Acad. Sc. Paris, Vol. 95.
3. Blochmann, F., 1887, Ueber die Richtungskörper bei Insekteneiern, in: Morphol. Jahrb., Vol. 12.
4. —, 1889, Ueber die regelmäßigen Wanderungen der Blattlaus, in: Biol. Ctrbl., Vol. 9.
5. Baumgartner, W. J., 1902, The spermatid transformations of Gryllus assimilis, in: Kansas Univ. Sc. Bull., Vol. 1, No. 1.
6. —, 1904, Some new evidences for the individuality of the chromosomes, in: Biol. Bull., Vol. 8, No. 1.
7. Brass, A., 1883, Das Ovarium und die ersten Entwicklungsstadien des Eies der viviparen Aphiden, in: Ztschr. Naturwiss., Vol. 55.
8. Flogel, J. H. L., 1904, Monographie der Johannisbeeren-Blattlaus, Aphis ribis L. (mit 9 Figuren), in: Allg. Zeitschr. Entomol., Vol. 9.
9. —, 1905, dsgl., ibid., Vol. 9.
10. —, 1905, dsgl., ibid., Vol. 1.
11. Henking, H., 1888, Ueber die Bildung von Richtungskörpern in den Eiern der Insecten und deren Schicksal, in: Nachr. Ges. Wiss. Göttingen.
12. —, 1891, Untersuchungen über die ersten Entwicklungsvorgänge in den Eiern der Insekten, in: Z. wiss. Zool., Vol. 51.
13. Huxley, T. H., 1858, On the agamic reproduction and morphology of Aphis, 2 parts, 5 plates, in: Trans. Linn. Soc. London, Vol. 22.
14. Korschelt, E., 1886, Ueber die Entstehung und Bedeutung der verschiedenen Zellenelemente des Insektenovariums, in: Z. wiss. Zool., Vol. 43.

15. LUBBOCK, J., 1851, On the ova and pseudova of Insects, in: Phil. Trans. Roy. Soc. London, Vol. 149.

16. METSCHNIKOFF, E., 1866, Untersuchungen über die Embryologie der Hemipteren, in: Z. wiss. Zool., Vol. 16.

17. MCCLUNG, C. E., 1900, The spermatocyte divisions of the Acrididae, in: Kansas Univ. Quart., Vol. 9, No. 1.

18. MONTGOMERY, T. H., 1904, Some observations and considerations upon the maturation phenomena of the germ cells, in: Biol. Bull., Vol. 6.

19. PHILLIPPS, E. F., 1903, A review of parthenogenesis, in: Proc. Amer. phil. Soc., Vol. 42, No. 174.

20. SUTTON, W. S., 1900, The spermatogonial divisions of Brachystola magna, in: Kansas Univ. Quart., Vol. 9.

21. STEVENS, N. M., 1905, A study of the germ cells of Aphis rosae and Aphis oenotherae, in: Journ. exper. Zool., Vol. 2, No. 3.

22. STSCHELKANOVZEW, J. P., 1904, Ueber die Eireifung bei viviparen Aphiden, Aphis rosae, in: Biol. Ctrbl., Vol. 24, No. 3.

23. WITLACZIL, EM., 1884, Entwicklungsgeschichte der Aphiden, in: Z. wiss. Zool., Vol. 40.

24. —, 1882, Zur Anatomie der Aphiden, in: Arb. zool. Inst. Wien, Vol. 4.

25. WHEELER, W. M., 1893, A contribution to Insect embryology, in: Journ. Morphol., Vol. 8.

26. —, 1889, The embryology of Blatta germanica and Doryphora decemlineata, ibid., Vol. 3.

27. WEED, C. M., 1890, Second contribution to the knowledge of certain life histories of certain little known Aphidae, in: Psyche, Vol. 5, No. 157/159, p. 208.

28. WILL, LUDWIG, 1888, Entwicklungsgeschichte der viviparen Aphiden, in: Zool. Jahrb., Vol. 3, Anat.

29. —, 1883, Zur Bildung des Eies und des Blastoderms bei den viviparen Aphiden, in: Arb. zool.-zoot. Institut Würzburg, Vol. 6.

30. WILCOX, E. V., 1895, Spermatogenesis of Caloptenus femur-rubrum and Cicada tibicen, in: Bull. Mus. comp. Zool. Harvard Coll., Vol. 28, No. 1.

31. —, 1896, Further studies on the spermatogenesis of Caloptenus femur-rubrum, ibid., Vol. 29.

Explanation of plates.

Plate 49.

Fig. 1. The spireme stage of the last ovogonial division.

Fig. 2. The division of spireme in the formation of chromosomes.

Fig. 3—4. Formation of chromosomes, 4 large, 2 small.

Fig. 5—8. The last ovogonial division.

Fig. 9. Polar view of last ovogonial division.

Fig. 10—11. Beginning of the growth period and appearance of chromosomes.

Fig. 12. Cross section of the distal end of the end chamber with ovum at the time of its entrance into the oviduct.

Fig. 13. Long section of ovum, showing the distinct germinal vesicle membrane and the granular appearance of the chromatin. *y. s* yolk spheres.

Fig. 14. Ovum showing nucleus in synapsis.

Fig. 15. Long. section of part of an ovum showing condition of germinal vesicle in center, at the end of the growth period. *pr* periplasm, *y. s* yolk spheres.

Fig. 16—17. Section of germinal vesicle only, showing condition of nucleoplasm and the formation of the chromatin spireme. *ncl* nucleolus.

Fig. 18—19. Division of spireme and formation of chromatin rod or chromosomes.

Fig. 20. Longitudinal splitting of chromosomes.

Fig. 21. Long. section of ovum at the time of the formation of the first polar body. pb_1 first polar body, *ncl* nucleolus.

Fig. 22—23. Condition of o[illegible] after the formation of the second polar body. [illegible], *p* pronucleus.

Fig. 24. Later stage than preceding, showing granular appearance of the polar bodies and pro-nucleus, just before the breaking down of the wall of the germinal vesicle. *prn* pronucleus, *y. s* yolk spheres.

Fig. 25. Long. section of ovum showing the fusion of the nucleoplasm with the periplasm. *pbs* polar bodies, *prn* pronucleus.

Fig. 26. Tangential section a little later than preceding, showing the spreading of the nucleoplasm. *prn* pronucleus, pb_2 second polar body, *pr* periplasm.

Fig. 27. Condition of ovum with polar bodies scarcely visible and the pronucleus moving toward the interior of egg.

Fig. 28. Pronucleus in center of the ovum.

Plate 50.

Fig. 29. Long. section of follicle of a half-grown embryo. *e. ch* end chamber, *o. gl* ovarian glands, *ov* ova, *n. s* nutritive string.

Fig. 30. Section of follicle at time of birth, showing last ovogonial division. *ovg* ovogonia; lettering same as preceding.

Fig. 31. Transverse section of follicular epithelium at base of end chamber, showing an ovum during growth, and the origin of the true ova from the follicular cells. *oog* ovogonia, *f. e* follicular epithelium.

Fig. 32. Condition of follicle at the beginning of deposition, showing the exact relation of the ovarian glands, nutritive string and ovum. *ov* ovum.

Fig. 33. Section of follicle a little later than preceding, containing a completely formed ovum and showing the condition of the ovarian glands near the end of deposition.

Fig. 34—40. Cleavage and formation of the blastoderm in the asexual forms. *pb* polar body, *clv* cleavage nuclei, *bl* blastoderm.

Fig. 41. Long. section of egg with completely formed blastoderm, at the beginning of invagination. Invagination first begins by proliferation of cells at posterior pole. *pc* proliferating cells of blastoderm, *pr. y* primary yolk, *el* end ligament.

Fig. 42. Section a little older than preceding showing entrance of secondary yolk into the interior. *s. y* secondary yolk.

Fig. 43. Long. section of a single follicle of asexual female. *e. l* end ligament, *o. gl* ovarian glands, *ov* ovum.

Fig. 44. Transverse section of a single cyst showing the prophase of the spermatocyte of the first order.

Plate 51.

Fig. 45. Long. section of a single lobe of the testis, showing the male cells in different stages of development. *a* anterior end, *p* posterior, sp_1 spermatocytes of first order, sp_2 spermatocytes of second order.

Fig. 46. Long. section of the posterior part of a sexual ovum, showing the entering of follicular cells from the follicular walls of the oviduct which form the secondary yolk.

Fig. 47. Transverse section of the extreme posterior end of sexual ovum, showing the transformation of the follicular cells into the secondary yolk. *f. c* folliculor cells, *s. y* secondary yolk.

Fig. 48. Transverse section of a single ovarian gland. *k* karyosomes, *cy* cytoplasm, *ch* chromatin with denser portion of nucleoplasm.

Fig. 49. Long. section of posterior end of ovum, with the early proliferation of the blastoderm cells. *bl. c* blastoderm cells, *s. y* secondary yolk, *y. s* yolk spheres or primary yolk.

Fig. 50. Long section of sexual developing embryo at the beginning of gastrulation. *bl* blastoderm, *s. y* secondary yolk.

Fig. 51. Longitudinal section of developing embryo when the germ band is entirely immersed and separated from uninvaginated part of the blastoderm. *s* serosa, *g. b* germ band, *am* part of the germ band which becomes the amnion, *s. y* secondary yolk.

Plate 52.

Fig. 52. Long. section of posterior end of egg, showing the portion of the blastoderm which becomes the germ band during gastrulation. *bl* blastoderm, *s. y* secondary yolk, *s* serosa.

Fig. 53. Long. section of egg with male and female pronucleus before fusion.

Fig. 53a. Pronuclei a little later than preceding.

Fig. 54a. Fusion of nucleoplasm and periplasm. *np* nucleoplasm, *pr* periplasm, *p. bs* polar bodies, *prn* pronucleus.

Fig. 54—60. Cleavage and formation of the blastoderm. *Cl. c* cleavage cells, *s. y* secondary yolk, *y. n* yolk nuclei derived from cleavage cells, *bl* blastoderm.

Fig. 61. Stage a little later than preceding showing the beginning of the proliferation of blastoderm cells at the posterior pole.

Plate 53.

Fig. 62—74. Spermatogonia before and after the last spermatogonial division.

Fig. 75—80. Growth of spermatogonia and formation of spireme.

Fig. 81—97. Formation of chromosomes and first spermatocytic division.

Fig. 98—106. Second spermatocytic division.

Fig. 107—110. Spermatids at the beginning of transformation period.

Fig. 111—116. Degenerating male cells.

Fig. 117—119. Ovogonia of the asexual forms, from the beginning to the end of growth period.

Fig. 120. Condition of ovum before the breaking down of the germinal vesicle.

Fig. 121—125. Formation of the polar body. *p. b* polar body.

Fig. 126—130. Behavior of pronucleus and first cleavage.

Lippert & Co. (G. Pätz'sche Buchdr.), Naumburg a. S.